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Effect of Ethylene Antagonists on Auxin-induced Inhibition of Intact Primary Root Elongation in Maize (*Zea mays* L.)

Kim, Soon Young* and Timothy J. Mulkey[1]

Department of Biology, Andong National University, Andong 760-749, Korea

[1]Department of Life Sciences, Indiana State University, Terre Haute, IN 47809, USA

Root elongation was measured in intact primary roots of maize (*Zea mays* L.) using a computerized root auxanometer. We examined the fact that root elongation was inhibited by auxin-induced ethylene production. Inhibition of root elongation was closely correlated with the concentrations of the exogenously applied auxin. Auxin-induced inhibition of root elongation was reversed by pretreatment or posttreatment of ethylene biosynthesis antagonists such as aminoethoxyvinylglycine (AVG) and silver ions (Ag^{2+}). The magnitude of recovery effect was dependent on auxin concentrations. Root elongation was inhibited by adding ethylene producing agents such as 1-aminocyclopropane-1-carboxylic acid (ACC) and Ethephon. ACC- and Ethephon-induced inhibition of root elongation was reversed by blocking ethylene biosynthesis or activity. These data suggested that ethylene was involved in auxin-induced inhibition of root elongation and inhibition of root elongation by applied auxin, at least in part, was a reversible process.

Keywords: *Zea mays* L., auxin, ethylene, intact primary root, elongation rate

Many researchers have attempted to demonstrate that auxin stimulates root growth in a concentration-dependent manner. This idea originated with Thimann's experiment (Thimann, 1937). He suggested that low concentrations of auxin promote root growth in a manner similar to the effect of high concentrations of auxin on stem or coleoptile growth.

However, attempts to demonstrate auxin stimulation of root growth have been inconsistent. The possibility is that auxin-induced ethylene production interrupts the action of auxin in root growth. Auxin induces ethylene biosynthesis in roots (Mulkey *et al.*, 1982; Kim and Mulkey, 1988). Ethylene is a potent inhibitor of root growth (Whalen and Feldman, 1988). Mulkey *et al.* (1982) reported that auxin-induced promotion of ethylene biosynthesis mediated the effect of auxin on primary root elongation of *Zea mays* L. However, Eliasson *et al.* (1989) reported that auxin-induced inhibition of root growth in pea was caused by auxin *per se* based on the measurements of root growth and swelling measured as fresh weight of root tips after 24 h in the presence of growth regulators such as IAA and ACC. And they measured ethylene production in the excised root tip which was grown for 24 h in the presence of IAA or ACC.

In this experiment, we measured root elongation every 5 sec as mentioned in Materials and Methods instead of 24 or 48 h. Therefore, our system is appropriate to detect any small changes of root elongation clearly and exactly. We examined the hypothesis that auxin-induced ethylene biosynthesis inhibits root elongation within relatively short periods (2-8 h) using a computerized root auxanometer in intact primary roots of maize. Also, this study investigated whether inhibition of root growth by high concentrations of auxin can be reversed by inhibitors of ethylene biosynthesis or ethylene action.

MATERIALS AND METHODS

Plant Material

Maize (*Zea mays* L., Pioneer 3343) seeds were soaked overnight in running tap water and germinated between wet paper towels on opaque plastic trays placed in a vertical position (Mulkey *et al.*, 1981). The trays were kept in a growth chamber at 27°C. Little light reached the seeds and the seedlings were used after 1.5 d when the primary roots were about 15 to

*Corresponding author: Fax +82-571-841-1627

20 mm long.

Measurement of Root Elongation

Root elongation was measured using a computerized root auxanometer similar to ones previously described (Evans, 1976; Mulkey *et al.*, 1982). The roots were placed in the auxanometer chamber containing half-strength Meyer's solution, pH 6.5. The solution was continuously oxygenated. The root auxanometer utilized a Schaevits model R30D linear variable differential transformer (Schaevits engineering, Pennsauke, NJ, USA). The voltage output from the transformer was directed to a Personal Computer after analog to digital conversion of the signal (Dash 8/EXP 16 converter and multiplexer, metrabyte Corp. Stoughton, MA, USA). Resolution was ± 2 μm with a repeatability of $\pm$ 3 μm. Rate measurement were stored every 5 s for further analysis. Elongation rate was calculated on the basis of change in relative length over time periods of 5 and 60 s.

Pretreatment of Root

Primary roots were pretreated with inhibitors of ethylene biosynthesis or action or with the desired chemical solution, depending on experiments. Roots were placed vertically in an oxygenated aqueous solution containing testing compounds for proper time periods. Roots were transferred to the auxanometer for measurement of root elongation.

Chemicals

Ethephon was purchased form Carolina Biological Supply Company (Burlington, NC, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA). To observe the effect of silver ions (Ag^{2+}), silver thiosulfate (STS) was prepared with silver nitrate and sodium thiosulfate. Sodium thiosulfate was dissolved in distilled water at eight times the final concentration of silver nitrate. After the sodium thiosulfate was completely dissolved, the silver nitrate was added very slowly with continuous and vigorous stirring. The solution changed color to a brown liquid without a precipitate if the silver nitrate was added slowly. The resulting silver thiosulfate solution was used immediately.

RESULTS

In Fig. 1, the dotted line represents the growth kinetics associated with treatment of roots with prior to exposure to IAA. After a steady growth rate was observed, the roots were treated with 10^{-6} M AVG. Addition of 10^{-6} M IAA inhibited root elongation by about 90%. The solid line illustrates the effect of the converse of treatments described above. Roots were treated with 10^{-6} M IAA. This resulted in inhibition of root elongation to 90% within 30 min. Root elongation recovered to approximately 60% of the control rate within 2 h.

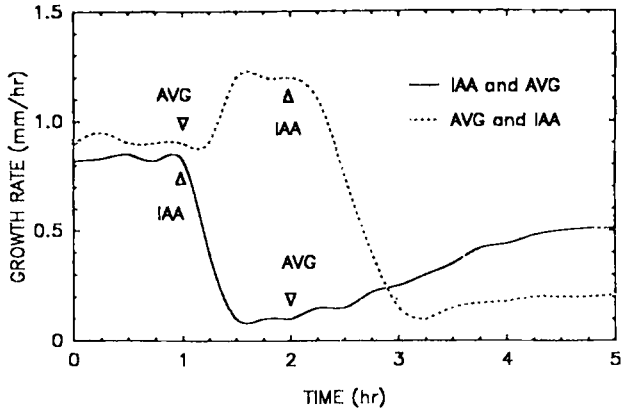

Fig. 1. Effect of 10^{-6} M AVG and 10^{-6} M IAA on the rate of elongation of intact primary roots of maize. Solid line illustrates the effect of treatment with 10^{-6} M IAA (first arrow) and application of 10^{-6} M AVG (second arrow) on root elongation. Dotted line is the converse treatment. Note the recovery from IAA-induced inhibition after AVG treatment.

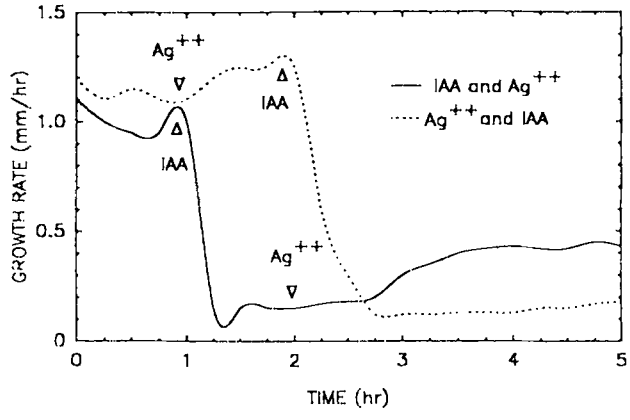

Fig. 2. Effect of 10^{-4} M Ag^{2+} and 10^{-6} M IAA on the rate of elongation of intact primary roots of maize. Solid line illustrates the effect of treatment with 10^{-6} M IAA (first arrow) with subsequent exposure to 10^{-4} M Ag^{2+} (second arrow) on root elongation. Dotted line is the reverse treatment. Note the recovery from IAA-induced inhibition after Ag^{2+} treatment.

Treatment of primary roots with 10^{-4} M Ag^{2+} promoted elongation (Fig. 2, dotted line). This promotion by Ag^{2+} suggested that intact roots produce ethylene without externally applied IAA. Thus, root elongation might be dampened or modulated by ethylene produced by the root. Addition of 10^{-6} M IAA inhibited elongation by approximately 85% within 30 min. The solid line in Fig. 2 showed the reverse treatments of those as described above. Root elongation was inhibited by 90% when intact primary roots were treated with 10^{-6} M IAA as shown in Fig. 1 and Fig. 2. This inhibition of elongation by IAA could be reversed with subsequent treatment with 10^{-4} M Ag^{2+}. The treatment of auxin inhibited roots allowed recovery of the elongation rate to 40% of the control rate. The recovery effect was observed 45 min after Ag^{2+} treatment. This recovery effect was maintained for a minimum of 2 h.

In order to examine the effect of ethylene on root elongation, roots were treated with 10^{-3} M Ethephon, an ethylene producing agent, at > pH 5. Application of 10^{-3} M Ethephon stimulated elongation considerably without a lag period. The root elongation rate declined toward the control rate 1 h after Ethephon treatment. This stimulation by Ethephon is due to acidification of the solution surrounding the root. This temporary acidification of the solution resulted in a transient acid growth effect. After the acid stimulation of growth during Ethephon decomposition, the elongation rate was inhibited by 80%. Application of 10^{-4} M Ag^{2+} to Ethephon-inhibited roots allowed for recovery of root elongation to the control rate (Fig. 3).

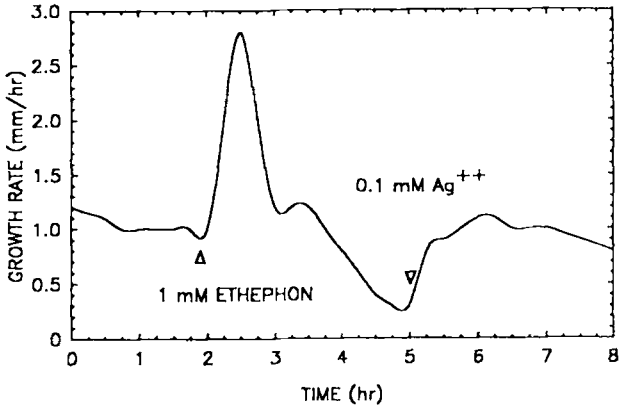

Fig. 3. Effect of 10^{-4} M Ag^{2+} on the 10^{-3} M Ethephon-induced elongation rate of intact primary roots of maize. Roots were treated with 10^{-3} M Ethephon after steady growth was observed (first arrow). Sliver ion (10^{-4} M) was applied (second arrow) to the root after elongation was inhibited by Ethephon.

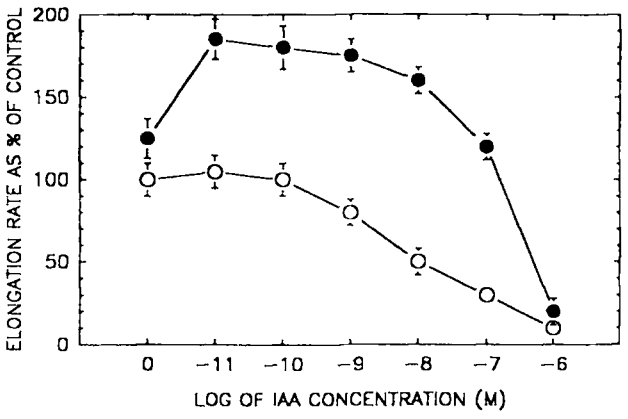

Fig. 4. Concentration dependence of IAA action on the rate of elongation of intact primary roots of maize. Open circles are elongation rate of roots pretreated with control medium which was half-strength Meyer's solution without 10^{-6} M AVG for 1 h. Closed circles are elongation rate of roots pretreated with 10^{-6} M AVG for 1 h. Elongation rates were measured 2 h after IAA treatment.

IAA exerted an inhibitory effect on root elongation which was concentration dependent (Fig. 4). The rate of elongation was mildly inhibitory at 10^{-9} M IAA, and was severely inhibitory at 10^{-6} M IAA (open circles). At 10^{-11} M and 10^{-10} M, IAA was not inhibitory to root elongation. Roots pretreated with AVG for 1 h prior to exposure to IAA exhibited promotion of root elongation at concentrations of IAA from 10^{-11} M to 10^{-7} M IAA (closed circles). AVG pretreated

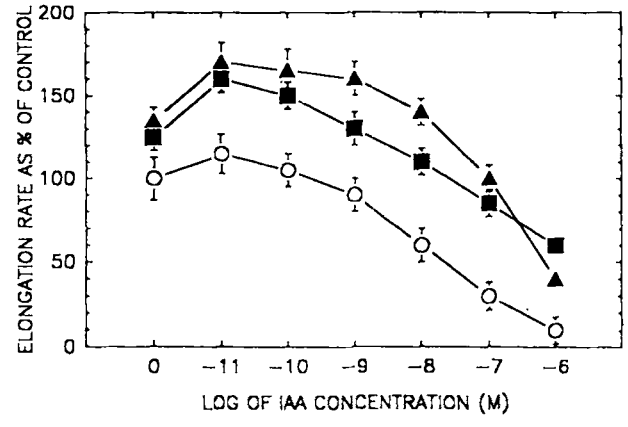

Fig. 5. Concentration dependence of pretreatment with IAA on the rate of elongation of intact primary roots of maize. Roots were pretreated with IAA for 1 h. Circles are elongation rate of roots treated with control growth medium. Squares are elongation rate of roots treated with 10^{-6} M AVG. Triangles are elongation rate of roots treated with 10^{-4} M Ag^{2+}. Elongation rates were measured 2 h after treatment with AVG or Ag^{2+}.

roots exposed to high concentrations of IAA (10^{-6} M) exhibited strong inhibition of root elongation as previously illustrated in Fig. 1.

Fig. 5 showed the effect of AVG and Ag^{2+} posttreatment on IAA-treated roots. Roots were pretreated with IAA for 1 h. Subsequently 10^{-6} M AVG (squares) or 10^{-4} M Ag^{2+} (triangles) was added. AVG or Ag^{2+} posttreatment of IAA-treated roots induced recovery from IAA-induced inhibition. However, the difference in amount of recovery was dependent upon IAA concentrations. Roots treated with 10^{-6} M IAA showed recovery after treatment with AVG or Ag^{2+} (Fig. 1 and Fig. 2).

DISCUSSION

High concentrations of auxin (e.g. 10^{-6} M) usually inhibit root growth. The magnitude of inhibition is closely correlated with the concentration of the exogenously applied auxin. Even though there were reports which demonstrated promotion of root growth at very low concentrations of auxin, it was difficult to demonstrate auxin-induced portion of root elongation. One factor which complicates the study of auxin action is that auxin rapidly increases ethylene levels in plant tissues (Abeles *et al.*, 1992). To explain auxin action on root growth, it has been suggested that roots maintain optimal or supraoptimal concentrations of auxin (Kim and Mulkey, 1988) and any additional auxin stimulates auxin-induced ethylene production (Mulkey *et al.*, 1982).

Pretreatment of roots with an ethylene biosynthesis inhibitor (AVG) and an ethylene action inhibitor (Ag^{2+}) (dotted line in Fig. 1 and Fig. 2, respectively) promoted root elongation. This stimulation of growth suggests that "normal" auxin levels in root tissue are supraoptimal; thus suppression of ethylene biosynthesis or ethylene action which results from the endogenous auxin levels allows for the enhancement of root elongation. The results illustrated in Fig. 1 and Fig. 2 demonstrated the recovery effects of AVG and Ag^{2+} on root elongation which was induced by high concentrations of IAA (10^{-6} M). The effects of posttreatment with AVG suggested that inhibition of root elongation by IAA results from auxin-induced inhibition of root elongation was also observed in the roots treated with Ag^{2+}, suggesting blocking of ethylene action stimulates root elongation. Eliasson *et al.* (1989) suggested that auxin induced inhibition of root elongation was not mediated by ethylene. They observed root elongation and root tip swelling in the presence of IAA after 24 h or 48 h. The results showed that IAA inhibited root elongation at 24 h or 48 h, but inhibited ethylene production at these time duration. Nevertheless, they observed that IAA increased ethylene production within 5 h. However, IAA would be metabolized in such a long time by IAA oxidase. As our results presented in here, IAA-induced inhibition of root elongation occurred within 30 min.

Ethylene treatment in the form of Ethephon clearly demonstrated the relationship between ethylene and root elongation. There was an evidence that inhibition of root elongation by treatment of ethylene was reversible (Whalen and Feldman, 1988). They reported that inhibition of root elongation by treatment of ethylene took place within 20 min, and elongation recovered to control values within 15 min after ethylene was removed. In this experiment, root elongation was inhibited by ethylene treatment; but this inhibition of root elongation was reversed by Ag^{2+} (Fig. 3). These results suggested that auxin-induced inhibition of root elongation resulted from auxin-induced ethylene production. Furthermore, the recovery response observed after exposure of auxin- or Ethephon-inhibited roots to Ag^{2+} supported the conclusion that auxin in root elongation is related to auxin-induced ethylene production.

Pretreatment of primary roots of maize with AVG stimulated root elongation in the presence of several concentrations of auxin (Fig. 4). This stimulation was dependent on auxin concentrations except 10^{-6} M IAA. Mulkey *et al.* (1982) showed that Co^{2+}/AVG pretreatment promotes the root elongation in the presence of various concentrations of auxin. They also found that the stimulation of root elongation by Co^{2+}/AVG was not significant in the presence of 10^{-6} M auxin. Furthermore, Mulkey *et al.* (1982) examined auxin action on root elongation in regard to H^+ efflux. They found that the promotion of growth by low concentrations of auxin in Co^{2+}/AVG pretreated roots was associated with the enhancement of H^+ secretion. However, the non-pretreated roots exhibited no increase in elongation and no observable H^+ secretion in the presence of low concentrations of auxin. High concentrations of auxin (e.g. 10^{-6} M) inhibited growth and promoted H^+ uptake in pretreated and control roots. These data suggest that one of the rapid auxin actions on roots may be mediated by H^+ secretion or uptake (Mulkey *et al.*, 1982).

Posttreatment of intact roots with AVG and Ag^{2+} allowed for the recovery of the elongation rate in roots which were previously exposed to various concentrations of auxin including 10^{-6} M IAA. These data demonstrated that the inhibition of elongation by aux-

in could be partially (Fig. 1 and Fig. 2) or fully reversed (Fig. 3) by blocking ethylene biosynthesis or activity. These data provided further support for the involvement of ethylene in auxin-induced root inhibition. Furthermore, this study demonstrated that auxin inhibition of root elongation, at least in part, is a reversible process in intact primary roots of maize.

LITERATURE CITED

Abeles, F.B., P.W. Morgan and M.E. Saltveit, Jr. 1992. Ethylene in plant biology. Academic press, Inc., San Diego, CA.

Eliasson, L., G. Bertell and E. Bolander. 1989. Inhibitory action of auxin on root elongation not mediated by ethylene. *Plant Physiol.* **91**: 310-314.

Evans, M.L. 1976. A new sensitive root auxanometer: preliminary studies of the interaction of auxin and acid pH in the regulation of intact root elongation. *Plant Physiol.* **58**: 599-601.

Kim, S.Y. and T.J. Mulkey. 1988. Effects of ethylene antagonists on recovery from auxin-induced inhibition of root elongation. *Curr. Topics in Plant Biochem. Physiol.* **7**: 228.

Mulkey, T.J., K.M. Kuzmanoff and M.L. Evans. 1981. Correlations between proton efflux patterns and growth during geotropism and phototropism in maize and sunflower. *Planta* **152**: 239-241.

Mulkey, T.J., K.M. Kuzmanoff and M.L. Evans. 1982. Promotion of growth and shift in the auxin dose/response relationship in maize roots treated with the ethylene biosynthesis inhibitors, aminoethoxyvinylglycine and cobalt. *Plant Sci. Lett.* **25**: 43-48.

Thimann, K.V. 1937. On the nature of inhibition caused by auxin. Amer. *J. Bot.* **24**: 407-412.

Whalen, M.C. and L.J. Feldman. 1988. The effect of ethylene on root growth of Zea mays seedlings. *Canadian J. Bot.* **66**: 719-723.

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